Supporting Information

Quantitative profiling of integrin αvβ3 on single cells with quantum dot labeling deeply revealed phenotypic heterogeneity of glioblastoma

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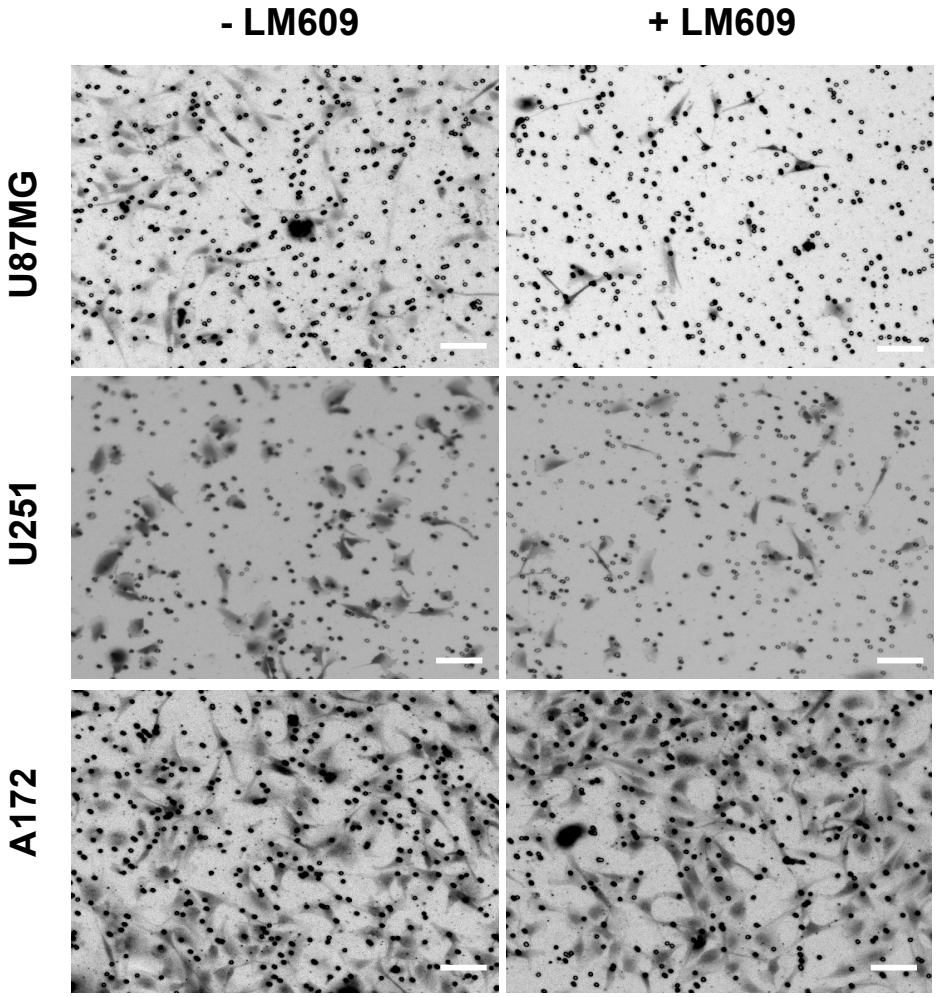

Figure S1. Representative images of transwell invasion assay (914 × 723 μm/field). The cells were stained with Crystal Violet, and the images were taken by Cytation 3 (BioTek, Gene Company Limited, Hong Kong). Scale bars: 100 μm.

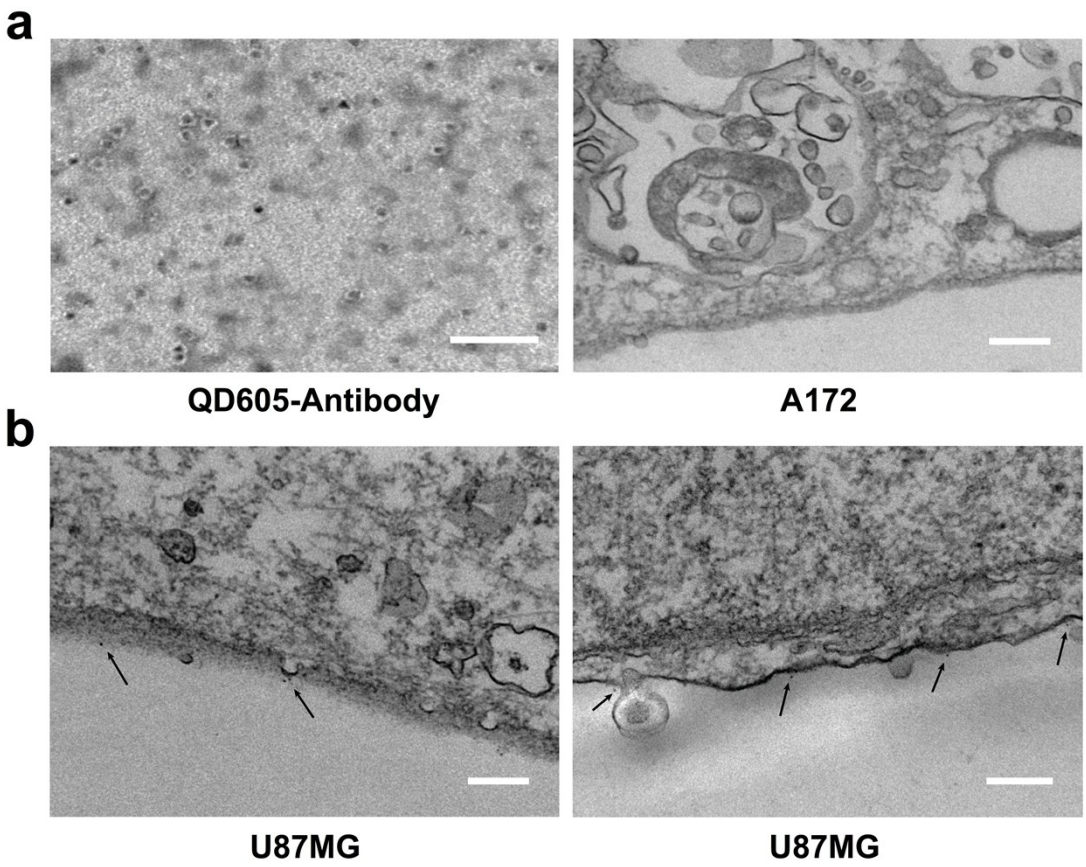

Figure S2. TEM imaging. a) TEM image of the QD-second antibody conjugates (left). Right shows the TEM image of the αvβ3 negative cell (A172), there is no QD-second antibody conjugates. b) TEM images show that QD-second antibody conjugates bond on the αvβ3 positive cell (U87MG) surface. All the cells were first incubated with the primary antibody, and then labeled with QD-second antibody conjugates. Scale bars: QD605-Antibody, 100 nm; others, 200 nm.

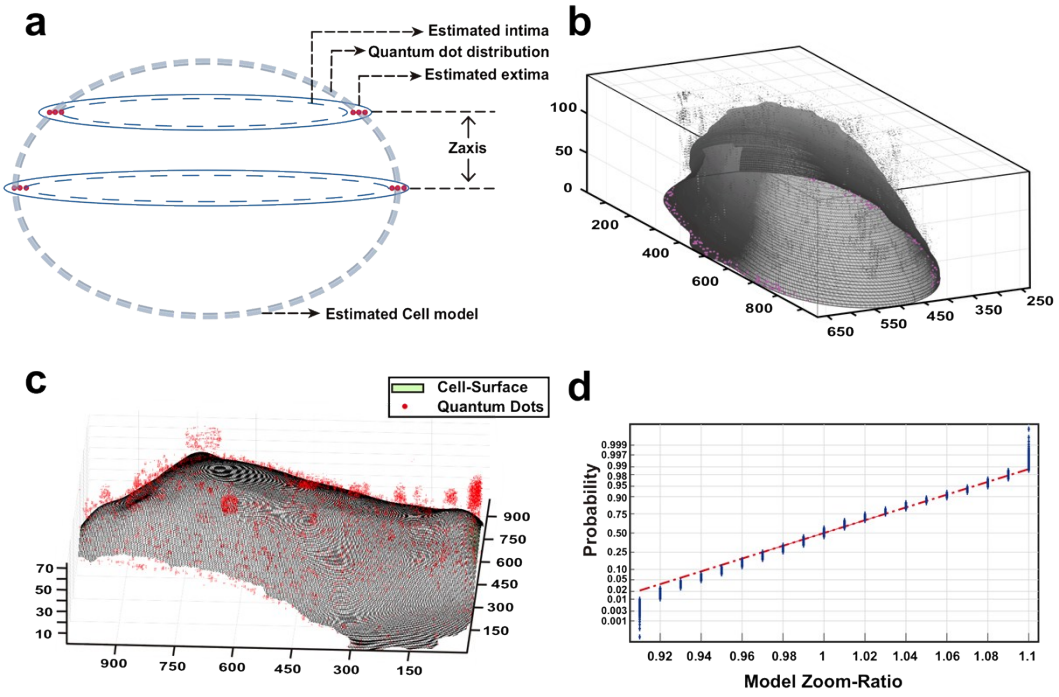

Figure S3. Validation of reconstructed local cell-surface. (a) Schematic diagram of smoothing the quantum-dots contour edge of each selected optical slice to reconstruct the surface. (b) Co-localization presented for the global perspective to obtain the distribution information of quantum dots (pink) around the reconstructed surface. The outline gap is the longitudinal section of the field of view. (c) Microvesicles-like (red clusters) from the U87MG cell line obstruct the progress of cell-surface reconstructions and therefore were not counted. (d) Normality of the probability density distribution of quantum dots around the reconstructed local cell-surface. After transforming the 3D surface with a different zoom-ratio [0.9, 1.1], we can obtain a series of numbers of quantum dots at different Euclidean distances to the reconstructed local cell-surface for the probability density estimation in the radial direction, which determines the entire distribution of quantum dots and can be used to validate if quantum dots are around the reconstructed local cell-surface. Here, zoom-ratio is the

proportion of the assumed polar radius to the actual polar radius, and the polar radius in each slice is the Euclidean distance between the contour edge and the corresponding projective polar from the centroid of the whole convex hull (Related details in the *methods* part).

Movie S1. Movie of integrin αvβ3 on single U87MG cell surface. The integrin αvβ3 on U87MG cells was first incubated with the primary antibody, and then labeled with QD605-secondary antibody. The cells were imaged with Structured illumination microscopy (SIM). (Mp4)